

Session Summaries and Discussion*

Session Summaries

J. A. COTRUVO: In the course of this conference we have had sessions devoted to the toxicology, mechanisms of action, chemistry, epidemiology, and clinical experience associated with drinking water disinfectants. Each of the session chairpersons will, in turn, summarize the discussions in each group.

D. COURI (Mechanisms): I will limit my remarks to the session which dealt with direct effects of disinfectants in studies involving cats, rats, goats, monkeys, and man. There appears to be a common toxicity which emerges among these species which is referable to the oxidative capacities of the Cl compound disinfectants. These oxidative agents produce effects which ultimately impair red blood cell functions. This was demonstrated in the chemical, morphological, and physiological data. For example, the decrease in red blood cell levels of intermediates such as the decrease in red blood cell glutathione produced hemolysis and morphological alterations of red cells in each of the species mentioned (except man). There are at least subtle hints that the erythrocytic function in man may in fact be under some circumstances and treatments vulnerable in the same direction. It is important to emphasize that definitive effects occurred at very high doses, much higher than we would expect to see from water disinfection. Few, if any effects were observed at the low doses, i.e., at 1-5 or 10 parts per million, which we would expect to be used in water treatment plants. It is likely that a unifying hypothesis on the mechanism of action could be derived from the original reports of Heffernan and others at the USEPA (HERL) laboratory, where it was shown that very high levels of hydrogen peroxide could be generated in the red cell after treatment of animals with chlorite. It is well known that hydrogen peroxide produces damage to cellular elements. However, the biological system has the capability of compensating for these oxidative results. Therefore, the

serious effects seem to be reversible, and may be of a transient nature.

Ammar discussed preliminary studies on embryotoxicity. Certainly at high doses maternal deaths occurred after treatment by the intraperitoneal route, but the administration of high doses of disinfectant in the drinking water also produced some effects on the embryo and the fetus. Bercz has shown in the monkey a previously unreported effect of chlorine dioxide or its metabolites on thyroid function at a dose of approximately nine milligrams per kilogram, which may prove to be the most significant toxicity. This effect deserves further attention.

Calabrese's discussion concerned two mouse strains, one having sufficient, the other deficient glucose-6-phosphate dehydrogenase activity. There were no major differences in the sensitivity of the two strains. The application of the results to human glucose-6-phosphate dehydrogenase-deficient individuals is not clear, because the deficient mice still possessed a much higher activity than that observed in humans deficient in this enzyme. I think that Calabrese's attempt to use the goat for another species has drastic limitations, inasmuch as the goat's carbohydrate metabolism in the red blood cells is entirely different from that of other mammals. Again this needs careful consideration in terms of removing such confounding factors before applying the results to man.

Although it is difficult to arrive at a safe level for humans with any great degree of certainty, I think based upon the studies reported here that somewhere below one part per million chlorine dioxide might be acceptable and about five parts is perhaps a ceiling for total Cl compound disinfectant in drinking water.

M. ROGUL AND C. SONICH (Epidemiology): The first studies of chlorination and cancer in humans examined the relationship between general measures of water quality and cancer mortality rates. The purposes of these studies were not to establish causality or to derive risk estimates that could be used as the basis for drinking water regulations, but were exploratory measures in epidemiology that were used to identify suspect cancer endpoints and associate water types that should be investigated with more definitive epidemiological methods.

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The epidemiology studies that we have heard during the conference use the information of these earlier studies as the basis for their designs. That is, these studies were designed to both test the hypotheses that were generated during the earlier studies and to further explore the new ideas of cancer risks associated with drinking water contaminants. Contrary to the original exploratory studies, this generation of studies is based on newly collected information concerning cancer disease rates and water quality.

As would be expected in the progression of scientific research, the methodology of these studies was markedly improved over the original studies and was certainly more sophisticated. Two of the five studies that we have heard during this conference have used cancer incidence, and all of the studies have collected and examined much more information concerning extraneous factors such as the potential confounders: occupation, resident stability, smoking—things that might bias the results. Much more extensive information has also been collected concerning drinking water exposures. Thus, the results of these studies significantly add to our knowledge concerning the potential cancer risks of chlorination. However, since these studies still must be considered as first generation studies, they do leave a number of unanswered questions, as might be predicted.

Rather than being discouraged with discrepancies in these studies, we should be encouraged by the similarities; we should note them and exploit them to the fullest extent. The overall trend is that the risk of gastrointestinal cancers such as colon or rectal cancers is increased by exposure to chlorinated water. These similar findings should be further examined in light of the different methodologies, exposures and diagnostic criteria, in an effort to explain the differences. Hence, we should address and recognize the challenges that are involved with the exploration of low level cancer risks in the general population.

The purpose of our roundtable discussion was to determine the role of epidemiology in future assessment of the health risks associated with drinking water chlorination. We discussed and critiqued past studies in an effort to focus on specific questions indicating future research needs. If any conclusion was reached by the group, it was that based upon the epidemiology studies completed thus far, there is an association between chlorination of water and cancer.

The discussion was enthusiastic and positive in regard to past, present, and future research; pointing out deficiencies yet noting that in only seven years of studying this problem we have come quite

far and are moving in the right direction. (For example, the water hardness/coronary heart disease issue has yet to come this far.) The problem is complex and must be approached in steps, with attack on one objective at a time. While it is difficult to summarize such a spirited discussion, I will present a few of the main points which illustrate the consensus.

It is recognized that a causal relationship between chlorination of drinking water and cancer has not been established. But causality should not be expected at this point. The purpose of the phase I (first generation) studies was to add supporting evidence for the existing hypotheses. These studies were not intended to answer all questions but were designed as screening studies to determine which cancer site(s) is important.

The unifying thread between these studies is that drinking chlorinated water puts some stress on the human body that results in chronic disease. The studies most strongly indicate rectal cancer, but colon and bladder cancers have also been indicated.

Discussion focused on examining these studies to define the refinements necessary in designing future studies to answer additional questions (both within and outside of the field of epidemiology). The refinement predominantly discussed deals with exposure assessment. Categorization of chlorinated vs. unchlorinated or surface vs. ground does not provide sufficient information. More detailed and specific monitoring data are needed to include a variety of measurements necessary to more completely characterize water supplies. Mixtures of exposures will vary with source regardless of whether the source is chlorinated or unchlorinated, surface or ground. In this situation, it is necessary to better understand the chemistry of chlorination and its reactive by-products.

In addition, we also need to better understand the biology of chlorination, i.e., the direct effects of oxidant chemicals on the host. The biological modifications and adaptations associated with water chlorination need to be defined. Perhaps study populations should be stratified biologically, although the feasibility of doing so might be questioned. Finally, a better understanding of cancer, especially colon cancer, would contribute to efficient study design. Is there truly a difference in ascending, descending, and transverse colon cancers as related to drinking water chlorination?

Such questions outside of the field of epidemiology must be considered, but questions also arise within the field as well. Misclassification is often a problem in epidemiology studies. This is especially true when examining death certificates as done in the first generation studies. However, death certi-

ficates have been found to be 85% accurate when noting cancer as the leading cause of death. Nevertheless, the second generation studies are looking for associations with cancer incidence by using tumor registries. Since the cases will be living, more accurate cancer and exposure data can be obtained.

It has also been pointed out that the epidemiology studies arrive at low relative risks such that their importance is questioned along with any conclusions derived from them. However, although the strength of the association is weak and the risk to an individual is low, one must look at the number of people potentially affected by the disease. Thus, a small increase in risk can have a profound effect in terms of the number of lives at risk when the background rate is high, as is the case for colon cancer. The attributable risk is an important measure to consider in public health decisions.

In short, our discussion enabled us to identify important issues to consider in designing the next generation of studies, the analytical study. The first generation studies served an important function in generating questions for the epidemiologist as well as the chemist, biologist, toxicologist or any scientist interested in this issue. Perhaps one member of our group summed it up best by saying that he could salute the flag of chlorination (as a cancer risk factor) as long as he did not have to pledge allegiance for the rest of his life. We are moving in a general direction based upon results of the first generation studies and the questions generated from them. The results of the second generation studies along with biological, toxicological and chemical data will determine our next move, but for now, the emphasis should be placed on obtaining more and better monitoring data.

F. KOPFLER (Chemistry): The session began with a study presented by Carlson in which he was working with a known class of compounds. After treatment with chlorine he was able to identify many of the products and, account for almost 100% of the products formed. In this model system of reacting PAHs with chlorine at different pHs, it was found that at the lower pHs chlorination and oxidation reactions occurred and at a higher pH, on the other side of the pK values, oxidation predominated. He identified most of the products and found really only one compound that showed some interesting biological activity—more activity than the parent compound. That was the 1-methyl-4-chloronaphthalene. In addition in to the pH the chloride ion concentration is also very important, as it should be since chloride ion takes part in the equilibrium resulting when chlorine dissociates in water.

Completely different reactions occurred when the polynuclear aromatic hydrocarbons were in true solution as opposed to being in particulate form. This was borne out in some of the other talks when we discussed reactions of some of the humic substances. Such substances tend to exist anywhere from true solutions to colloidal to particulates in nature, depending on pH and perhaps other conditions.

Stevens pointed out that the aqueous chemistry and reactions of chlorine dioxide with a lot of model compounds has been well studied and reported. He reported on what most of us here were really interested in, those reaction products produced from uncharacterized substrates found in drinking water.

The work reported by Johnson supported Carlson's finding that at lower pHs more chlorination products are observed and at higher pHs he obtained oxygenation products along with the incorporation of a lesser amount chlorine. Haloform production is, however, increased at higher pH.

Stevens showed that the reaction of chlorine dioxide at high pH results in less chlorine incorporation into the organic substrate than occurs at the more acid pHs. Nevertheless under the same conditions, chlorine dioxide results in the introduction of less chlorine into the organic compounds and in all cases, far less production of trihalomethanes.

The presentations of Johnson and Watts point out that, really to solve some of these problems, newer analytical techniques are going to be required. We discussed double focusing high resolution mass spectrometry, field desorption mass spectrometry and several others, including negative chemical ionization. Further development of analytical techniques is essential.

Although many reaction products can be identified, as Glaze pointed out in his talk, the reaction mixture still contains high molecular weight, very polar material that does not appear to have changed that much even though a large number of reaction products have been identified. This background of the high molecular weight materials does not particularly change with ozonation. They're also still there after chlorination. Dr. Johnson pointed out that the myriad of products identified in the studies at North Carolina account for perhaps 8 to 14% of the original carbon, and the same thing with chlorine dioxide. We see many products, but the TOC (the total organic carbon) has not changed appreciably.

The one study reported on chloramines is really just in its beginning stages. We don't know much about even the simple inorganic mono- and dichloramines. We have a lot to learn from just studying

the kinetics and dissociation of the disinfectants; but most particularly the chloramines. This basic information is needed before we can really come close to predicting what any of our products of alternate disinfection are going to be.

J. F. BORZELLECA (Toxicology): In our session we addressed the potential adverse health effects of the trihalomethanes. We conducted two series of studies: those with mice and those with the rats.

The long-term studies in female mice (B6C3F1) that Jones described are lifetime studies that attempt to evaluate the carcinogenic potential. This study is in progress; it's not complete. It is a well-designed, and well-executed study. We are eagerly awaiting the results. In our program in Richmond, we are evaluating the general toxicity of these materials. We have determined acute oral LD₅₀ values and 14- and 90-day subchronic exposures. The materials were administered by gavage rather than in drinking water to permit exposure to high levels (their solubility is limited).

Our subchronic studies reconfirmed the liver as the target organ for the trihalomethanes. An effect on the spleen was also noted. Subchronically, chloroform appears to be the most toxic of the four halomethanes that were evaluated, bromoform the least. A degree of hyporesponsiveness or "tolerance development" was observed in several immunological endpoints, the humoral immunity and cell mediated immunity, and the functional activity of the RES. The spleen weight was decreased and there was a decrease in colony-forming cells. These effects were seen only at 14 days and not at 90 days, suggesting hyporesponsiveness or tolerance development.

The trihalomethanes administered orally did not affect DNA synthesis. Effects were seen only when the material was administered intratesticularly to the mice. This reflects the effectiveness of the blood-testicular barrier. Multigeneration reproduction studies were conducted using high doses. There were no adverse effects on any of the usual reproductive parameters through three generations.

There were few significant behavioral effects using a number of systems (including some developed in our laboratories). Nothing suggests a progressive neuropathy or neurotoxicity. Using the taste aversion paradigm, the effect of chloroform was noted at about 30 mg. A progressive hyporesponsiveness or tolerance development to the trihalomethanes was also observed.

It was reported that chloroform stimulated both ornithine decarboxylase activity and the RNA polymerase 1 activity in the mouse. These are involved in the early stages of the carcinogenic response. Jones' study with Osborne-Mendel rats is not complete. Levels up to 1800 ppm in drinking

water were used. This study should resolve the issue of carcinogenicity in rodents. The data are not available yet. There were early deaths in the mouse because they were not accepting the drinking water. There were very few deaths in the rats. Excellent survival among rats at the high levels of chloroform were noted.

Data presented suggests that chloroform is not an initiator but is probably a promoter. It was reported that hepatic ornithine decarboxylase activity in the rat was stimulated by chloroform. This stimulation of ODC decreased with time. ODC activity in the kidney was depressed. Apparent tolerance to chloroform was noted.

Based on detailed toxicological investigations involving doses that were orders of magnitude greater than maximum anticipated human daily consumption, and which were administered acutely or subchronically, it can be concluded that the trihalomethanes are not highly toxic. The hyporesponsiveness or tolerance development to the effects of THM were demonstrated in a number of systems including the immune system and behavior, and the synthesis of systems including the immune system and behavior, and the synthesis of hepatic ornithine decarboxylase in both the mouse and the rat. The THMs stimulate the synthesis of hepatic ornithine decarboxylase RNA polymerase and RNA polymerase activity. Chloroform appears to be a promoter and not an initiator of carcinogenesis. Many of the effects reported were seen only at high doses.

R. J. BULL (Biological Test Systems): I think there are a few things to which we can call attention. The first is that there is some reason to be concerned about the so-called nonvolatile portion of the organic material in drinking water. It is now clear that treatment with certain disinfectants increases the level of certain types of biologically active molecules. This was most convincingly demonstrated on the papers of Zoeteman et al. and Kool et al. The mutagenic activity observed is not going to be attributable to the trihalomethanes. One reason is the concentration technique employed would not particularly recover the trihalomethanes. A second reason is that the trihalomethanes have little or no activity in the test systems used. Third, the activity of the trihalomethanes would not be the direct acting type indicated in the results. So, there are a variety of biologically active compounds that are generated in this nonvolatile fraction by chlorination that can be identified as potential problems. That is a long way from saying that the risks due to those materials are high, low or intermediate. One of the major conclusions of this conference will have to be that toxicologists, chemists, and epidemiologists are going to have to focus on the overall

problem of disinfectant by-products. We should not get completely entranced by the idea that correlations of health effects with chlorination of drinking waters is a simple one involving only trihalomethane formation.

There were several other things that I do not remember being specifically articulated but can be derived from the presentations. In my own presentation, I failed to call attention to one parallel between the toxicological and epidemiological results. Isacson reported that there was a possible relationship between agricultural runoff and the incidence of cancer in the epidemiological studies. Ottumwa, Iowa was a city selected for study in our work for that reason in our five-city study. It was the only city to give rise to significant increases in tumor incidence.

So far I have been speaking about problems associated with chlorination. Here we are really just beginning to understand the identity of by-products and tend to focus our attention on individual products. However, we must also begin to recognize the more general problem; that the use of strong oxidants for purposes of disinfection alters the chemical nature of the materials that are natural background in any surface water. There is some concern with synthetic organic chemicals, but it seems that in surface waters much of our problem with chemicals in drinking water now involve and will continue to involve disinfection reaction products with natural background material. This is the source of the trihalomethanes, and it seems to be the source of a sizable portion of the so-called TOX (total organic halogen) in drinking waters.

One of the problems that must be addressed when taking a bioassay approach to the field is that these methodologies are not chemically specific. They are specific for a particular type of biological activity. There are variations in and among source waters and in the same source water at different times of the year. There seem to be differences in the amounts of total organic halogen produced over the course of a year. With surface water sources like the Ohio River, a few hours or days time can result in substantial changes in the synthetic chemical composition of the water.

Variability in biological responses are also encountered, so it becomes important to avoid placing a great deal of value to the results of one experiment. I think that Zoeteman and Kool indicated there was about a threefold variation in the level of mutagenic activity they were seeing in the Rhine and Meuse in the course of a year. Bioassay results are likely to give a misleading picture of this variability because they do not distinguish between different chemicals, but integrate levels of a particular biological activity potentially caused by a large number of

chemicals. Therefore, bioassay does not take the place of chemical analyses. Consequently, we must be as systematic as possible from both the biological and chemical standpoints in trying to characterize what's happening with processes such as disinfection. Efforts should be directed toward developing a fundamental understanding of the problem. Once the chemistry is better understood and model chemicals are examined for toxicological properties, we could then look at actual drinking waters with better tools and minimize the methodologic problems that invariably arise with the use of any sample concentration methodology applied to organic chemicals in drinking water.

Chlorinated phenols are by-products of chlorination which have been repeatedly identified in drinking water. Koller's presentation dealt with chlorinated phenols. 2-Chlorophenol and 2,4-dichlorophenol have been poorly characterized from a toxicologic standpoint. The 2,4,6-trichlorophenol has been shown carcinogenic in the NCI bioassay program. It is significant to note that Koller is beginning to observe some toxicological effects of 2-chlorophenol, indicating that there are individual chemicals within that mixture that possess some of the activities that we're concerned with. It is unlikely, however, that a chlorinated phenol would account for the direct-acting mutagenic effects; my guess would be those compounds would be indirect-acting if they are active at all.

The last talk dealt a development that could add a great deal of specificity to epidemiologic investigations—that is binding of carcinogenic and mutagenic chemicals to macromolecules. Utilizing this property provides a potentially elegant means of establishing relative contributions of active chemicals like carcinogens may have to human disease processes. I believe that if someone would get very clever with the approach of using hemoglobin alkylation as a dosimeter it would be possible to begin to weigh a variety of exposure to chemicals in an integrated way. Using this analytical tool, it would be possible to determine the relative level of exposure to a multitude of chemicals. Dr. Pereira's group has looked at the *in vivo* binding of 16 chemical carcinogens to hemoglobin. These chemicals included indirect, as well as direct acting chemicals. Therefore, the binding of carcinogens to hemoglobin is a fairly general phenomenon that could be extremely useful in sorting out confounding exposures in epidemiological investigations.

L. J. McCABE (Clinical Studies): We discussed two approaches, the epidemiological and clinical. One of the epidemiological studies was the more classical type where you dragged up available data from a great many years in the past such as Tuthill's and Moore's study, and the other was the

more elaborate clinical chemistry used in the study reported by Miday. These are examples of following up on an existing practice of chlorine dioxide used for disinfection and concluding that nothing life threatening seemed to be occurring, and that these practices probably could continue to be employed. Tuthill's study certainly would indicate that if you introduced this new practice of chlorine dioxide in a community, you might want to set up some kind of health surveillance to keep track of those few things that might show shifts. He did indicate that there were some population shifts using it over this short time period and alluded to the fact that these population means could continue to shift if it was carried out over a longer period of time. So those things might be incorporated into a new change if some community was going to follow through on using chlorine dioxide.

It's possible that these techniques of Tuthill and Miday could also or should very appropriately be used on a community that's been using chloramines, because most of the studies that were discussed earlier were pointing a finger at what happens when you use chlorination. So we do have some possibility of using the epidemiological approach on chloramines as was done with the chlorine dioxide.

The final paper was presented by Reitz, a representative from Dow. I don't know whether it was apparent to those of you from the water utilities, but the two approaches being used would really make quite a difference, I think, to you. The trihalomethane limits using the epigenetic approach would be something like 100 $\mu\text{g/l}$; if you used a genetic approach, it would probably be 2 $\mu\text{g/l}$. We have a factor of 50 apparently in between those two approaches to what might be considered as trihalomethane regulation.

J. A. CORRUVO: I think those summaries were excellent in focusing the essence of the last few days of discussion. The title of this session is in part at least, discussion of regulatory issues and I'd like to just mention a few words in that direction before we open the discussion to the floor.

The decisions that a regulator makes perhaps are made with a somewhat different perspective and with somewhat different motives than some of the motives that influence the work that you have heard. A regulator, of course, includes all of those factors in the decision process, the toxicology and the epidemiology that exists, and any other indications of risk that can be computed. At the same time the decision context is not necessarily the obvious one. It's something that is determined by what the Congress has written into the law that tells one how to treat the information that's available and tells one what kinds of factors have to be taken into consideration. In addition to all the

toxicology and the risk part of the equation, one also considers the burden of proof, the quality of evidence that exists, and the cost and the practicalities of whatever the control options might be.

As you know, some decisions have already been made. In 1979 there was a regulation established for trihalomethanes as indicative of contamination of drinking water resulting from chlorination. The question that we ask ourselves—and that I would hope to get some more guidance from this meeting—is, was that a sufficient regulation by establishing a maximum contaminant level for that limited group of substances? Did that in fact change the national risk picture substantially? We are assuming that in the United States now as more and more communities take steps to reduce trihalomethanes, drinking water risk factors should have been reduced to some degree and hopefully they'll be reduced further in the future. The question is, do the data indicate that it's necessary to take more drastic action or more costly action in the future?

There were also some peripheral decisions made in the course of that regulation and in an advisory sense relating to the alternate disinfections such as chlorine dioxide. The suggestion was made that the inorganic residuals of chlorine dioxide derived by-products should not exceed one-half of a milligram per liter in cases where it was used. There were also suggestions made about the appropriateness of using ozone in certain situations where there is high organic water and where the probabilities would be that difficulties and quality problems would be caused as a result of ozone. Obviously, as a result of that regulation there is a very substantial increase in the use of chloramines, at least as distribution system disinfectants. There are a great number of unanswered questions as to whether the fallout that occurred from that regulatory decision in fact results in an increased protection of public health or possible substitution of one set of risks with another set of risks.

So, my conclusion is that clearly we have a very confusing situation here. I'm not sure that we are ready to answer very many of those questions that I just asked. But in the context that we all operate—which is one of various kinds of pressures, or at least motivations, some of them being limited by financial considerations—just how much information is necessary before one can make a certain decision in this regulatory context? The pursuit of knowledge for the sake of knowledge is, of course, an admirable direction to take. The question is, in which areas should the limited resources that we have be expended? Where is the greatest potential for pay off? Will increasingly sophisticated and expensive study in a particular area in fact lead to some different set of conclusions

or some different set of decisions? If the relative risk of exposure to disinfection by-products, and for example, the relative cancer risk turns out to be 1.3 instead of 1.2, does that lead to a particularly different set of decisions? If the relative risk turned out to be 10 instead of 2, that might lead to some very different decisions!

So the question is, where do we draw the line; where do we direct the limited resources that we have; where is the best pay off going to be, and to what degree should we divide that resource pie by investigating this aspect of drinking water quality versus other aspects of drinking water quality and risks associated with them. These include problems such as cardiovascular disease risks, trace metal contamination problems due to corrosion, etc., and biological problems which are not resolved. We must still consider water quality problems from biological contaminants. Keep in mind that in those cases we can "count the bodies" whereas in this case of disinfection by-products risk we can't, and so there are many difficult decisions to be made.

So there are a lot of difficult choices to be made and we have to make them, and unfortunately there are restrictions. So we have to decide where to put the resources and where the best payoff of human health protection will be, even though there may be some areas of interest which will not be pursued as a result. So with that then, I will open the floor for questions.

Discussion

T. D. JOHNSON (Univ. N.C., Chapel Hill): I'd like to focus on a couple of things that seem to me to need balancing in looking at the problems that we face. Most of the meeting was focused on the issues of trihalomethanes because we found trihalomethanes were easy to measure and we therefore hung our hat very much on trihalomethane levels. This has given us a number of biases. One question that needs to be asked particularly is, are the chlorination by-products from natural aquatic humic materials, which are major by-products from woody tissue the problem, or are the chlorination by-products from biological by-products the problem?

I think Bull's comment that the city which showed the best correlation was the agriculture runoff city is particularly germane in this question, and I think that the question needs to be asked because we have focused so much of our effort on the woody tissue chlorination by-product because of the nice, easily measured chloroform. In particular, there was one paper which began to address this question of biological by-products: Scully's paper, which dealt with the natural organics issue. It seems to me that the organic nitrogen compounds

or other biological by-products as they are chlorinated are of major concern and I see very little of that type of work being done. Although the one study I am familiar with, the 5-chlorouracil I mentioned before, gave in a small rat study a negative teratogenic response, the fact is that 5-chlorouracil was taken up to the extent of 3 per genome in DNA, and that in itself I think is a rational basis for concern. So I personally would like to see more of this kind of effort.

The other kind of effort I think that needs to be mounted is the need to couple the toxicological, epidemiological and chemical studies together. As a chemist, although I'm surrounded by biologists in my department, I'm bothered by the fact that I don't know when I do my separation processes, which are necessary in order for me to get any meaningful results, that the fractions that I am actually spending my time on are the interesting ones. It's clear to me, I don't know how it is to the rest of you, that chlorination by-products are a concern and the problem to me is, which ones can we focus on? I think this coupling of the chemical studies with the necessary separations that we have to do as chemists with Ames tests and with toxicological tests are a needed factor. I would like to see more joint studies.

J. A. COTRUVO (U.S. EPA, Washington, D.C.): I think you made a good point, which I would like to emphasize and that's in the matter of the biological components in the water. Traditionally, public water systems get very excited when they have algal blooms in their waters because this adds a bad taste and causes a lot of consumer complaints and so they will do anything that they can to avoid that situation, including moving to other disinfectants that don't provide the taste. Well, what has not been perceived to date has been, in fact, the reason for that taste is a very substantial increase in the chemical loading of that water, and that many of the measures that are being taken to ameliorate the taste in fact further increase the chemical complexity of the soup that's been produced. Addition of larger amounts of chlorine or other oxidants may, in fact, compound the problem. So if there are risks from organic chemicals and drinking water it's reasonable to assume that during those periods of time when algal blooms are occurring and tastes are being added, additional chlorine is being added and the risks could be substantially increased. So it may be that a different perspective needs to be placed in the minds of water plant operators as to how to respond to those kind of situations.

R. J. BULL (U.S. EPA, Cincinnati): I would like to make one comment about Johnson's statements here: I think the biological testing part of the disinfection by-products product business needs to

be focused. In the case of potable wastewater reuse, the circumstance which you are talking about with the 5-chlorouracil is even more likely a problem. Just a very quick look at some data that have been generated around with some of the reuse plants or potential reuse projects around the country, one thing that stands out is the percentage of the organic material that's in the form of organic nitrogen. It is certainly higher in that circumstance than it is in most places where we draw our water from streams where the nitrogen is probably captured and utilized by microorganisms along the way.

K. P. CANTOR (Nat'l. Cancer Inst., Bethesda): One or two remarks on some of the items that have been brought up for discussion so far and partially in response to the question as to whether trihalomethane regulation might have public health benefits. There's one crucial piece of data which involves environmental measurements that we do not now have which would be very important to have—that is what is the association between levels of trihalomethanes and levels of the genotoxic substances that are below the surface of the water that are the rest of the iceberg. I think the answer to that question would be more readily forthcoming. And I see that as a very strong research need that has come to my mind anyway, as a result of the discussion in this conference.

The remark was disturbing in another sense too. I think this is a time for toxicologists and epidemiologists to learn a great deal from each other and can contribute to the direction that research is taking in the other's field.

J. STARA (U.S. EPA, Cincinnati): You know we have investigated water for many years, and I remember some years ago when they found some 700 compounds in Cincinnati's drinking water. I am surprised that you say that we do not know what compounds besides halomethanes are in chlorinated water or as by-products of chlorination. And my specific question is, did Koller say that trichlorophenol and pentachlorophenol have some relationship to carcinogenesis?

J. A. COTRUVO: On the statement about not knowing what the substances are in drinking water, I think that one of the major points of the chemistry part of this meeting is that we know some of the compounds that are in drinking water. We can identify several hundred or several thousand, but when you add that all together, you still account for only perhaps 10 or 15% of the total mass of chemicals in water; thus one of the questions is, how far should that be pursued? Will we really be able to make decisions on the basis of identifying additional substances, or is there some other approach that will lead us to a conclusion more quickly.

UNKNOWN: You said that the THM serves as an indicator, but don't you think there will be utilities treating just for the THMs and ignoring the total organic chlorine, I mean the way it's set up?

J. A. COTRUVO: The assumption is that it won't be possible to treat only for the THMs. In the course of reducing the THMs, as a natural consequence many other substances will be reduced too, unless the method of reduction is some very selective process. Offhand, I don't know what that selective process would be. Some people might say aeration, but even that removes some number of substances in addition to the THMs as well as chemically transforms some of the substances that are there, so I don't think there are any really unique processes that would selectively remove THMs and not touch the rest. So with the information that was available in 1979, that was the most reasonable course to take, fully conscious of the fact that it was intended as a surrogate for all of the aggregate of the substances that are in there.

R. J. BULL: I think that has at least partially answered the question about the identification of chemicals. A lot of them have been identified, but I think everyone that is here recognizes the fact that one thing that has been established in this meeting is that there is biological activity associated with those parts of the material isolated from water which are completely separable from those chemicals which are identifiable chemically. So I think in some ways that it looks like the submerged part of the iceberg is a larger problem than that involving known chemicals. The answer to Stara's question about Koller's paper is that he presented preliminary data, which indicated a trend in the direction of a cocarcinogenic effect with both 2-chlorophenol and pentachlorophenol.

J. A. COTRUVO: Just for the record, trichlorophenol came up active in the NCI bioassays last year.

J. STARA: My only comment is about these many other chemicals. It's true that we know almost nothing about some of them but we know something about many of them. We know we have some toxicologic or in some cases epidemiologic data on other chemicals besides trichloromethane.

R. J. BULL: Yes, we do. The biggest problem in that respect, I think, is that the amounts are so small once you get past the trihalomethanes so as to be of very questionable significance on an individual basis. I think that's the thing that we're trying to deal with.

J. A. COTRUVO: Usually well below parts per billion concentrations. The number of chemicals identified in drinking water is in the thousands now. There is such a small amount of each there and it's so variable. Data will show something is in the Ohio water today, but it's not there tomorrow. It

will be very hard, I think, to deal with that variability. These disinfection by-products result from the reaction of the disinfectant with these natural materials which are always there and probably fairly similar in all waters. It is hoped that we're going to be dealing with the majority of the organic material in water when we deal with these reaction products of the humic substances with the disinfectant or amines from biological origins. This is a situation that is peculiar to drinking water and is probably specific to drinking water as opposed to other problems we see with environmental chemicals. This is something which is pretty much a universal problem in drinking water, and I think it's important to address that one for that reason if for no other.

UNKNOWN: Given the data on the changes in the intestinal flora in response to the possible risk factors from large bowel cancer, e.g., decreases of bacterial diversity in individuals of high fat diet, has any work been done or planned to examine the effects of disinfectants or their by-products on intestinal flora? And I was very glad to hear that Rogul introduced himself as a microbiologist.

M. ROGUL (U.S. EPA, Washington, D.C.): I think it is a very complex question. I did have some pertinent experience, because at one time I worked as a veterinary microbiologist and the use of the mouse posed problem which is germane to this question. It was found in the early studies on radiation that many mice which were being raised commercially harbored *Pseudomonas aeruginosa*. When these mice were irradiated, their immunological barriers became attenuated. The pseudomonads proliferated, and thereby caused disease and deaths which ruined many experimental projects. It was later found that the addition of chlorine in the form of sodium hypochlorite or hydrochloric acid in mouse colony drinking water could effectively eradicate *P. aeruginosa* from these mice. To my knowledge this is the way that all commercially produced mice are reared. I think most researchers are probably dealing with mice that have been drinking highly chlorinated water for numerous generations, and it has a profound effect on the intestinal flora of the mice. We found that this type of chlorine treatment also seemed to eradicate pseudomonads from the nasal turbinates and middle ears of mice. In response to the last part of your question, I don't know of any research ongoing or planned at the EPA which would answer your question.

In addition, since most mice are raised on chlorinated water, and I understand that chlorine can be metabolized *in vivo* into trihalomethanes, I think it would be prudent to interpret experimental toxicology data with this in mind.

C. MANG (Buffalo Power and Water Admin.,

Saskatchewan, Canada): I have two questions on algal toxins, both relating to health effects research that either has been done or is under way on the subject of health effects of chlorination of blue-green algae or of algal toxins and their metabolites. Is anyone aware of studies along those lines? There are studies that I'm familiar with on chlorination of algae with the intent of demonstrating that there are by-products including trihalomethanes. But [is anything known] on the toxicity?

J. A. COTRUVO: If I recall correctly, one of the requests in the research strategy for this year does include studies of this sort but I don't know of anything that's underway right now. I guess we haven't heard of anything.

The next written question submitted is what epidemiological studies have been carried out relating to prolonged ingestion of carbon tetrachloride in drinking water and the possible effects of carbon tetrachloride on hepatic carcinogenesis.

C. SONICH (U.S. EPA, Cincinnati): When you say prolonged effects of carbon tetrachloride that's difficult to address, because carbon tetrachloride has not been routinely monitored in drinking water; so it's hard to assess effects due to prolonged exposures. We recently had the opportunity to study the effects of a carbon tetrachloride spill into the Ohio river. It was an assessment of an acute exposure to a relatively high dose. Seventy tons of carbon tetrachloride were spilled upstream from Cincinnati. We did a study looking at patients that were in the hospitals along the river during a time when we knew there were high concentrations of carbon tetrachloride in the drinking water compared to a time when we knew there was virtually no carbon tetrachloride in the drinking water. Kidney and liver function tests routinely performed on patients upon their admission into the hospital were evaluated. A dose/response relationship for creatinine, a kidney enzyme, was indicated. Controlling for disease, the number of patients with elevated creatinine levels was higher when the dose of carbon tetrachloride was higher in the drinking water. As the dose decreased downstream, so did the number of people with increased creatinine levels. This could signal an effect due to the ingestion of carbon tetrachloride or may be due to random chance. Dr. Bercz, would you like to comment on the toxicology study we designed to verify this finding?

J. P. BERCZ (HERL, U.S. EPA, Cincinnati): The study tried to reproduce the actual dosages experienced during the spill. By the administration of such water to rats we tried to explain the apparent human effects. We have attempted to identify renal lesions as well as hepatic lesions in a two-week exposure which approximately equates the time of

exposure in the human population. We could not detect any adverse effects on the animals. There was rather extensive workup of all the serum chemistry and hematology parameters, and there was none that we could identify as adverse.

UNKNOWN: Carbon tetrachloride is carcinogenic in the B6C3F1 mouse being used as a positive control in a number of the NCI bioassays of the chlorinated hydrocarbons. And when you say a high concentration in the Ohio River, it was not high compared to what a toxicologist administers to an animal to get an effect. The levels observed did not exceed a few hundred parts per billion at any water intake on the river, maybe 340 ppb or so in Huntington. Where there were larger populations at work, it was no more than 50 ppb to 100 ppb and then for just a short time, a day or so. The highest we were able to detect in just the drinking water was 280 parts per billion. You would not see much chemical changes or organ changes in any animal at these concentrations.

A. A. STEVENS (U.S. EPA, Cincinnati): You made the point that no epidemiological study is very neat. I've just gone over that data with a carbon tetrachloride spill from our station in Louisville, and although we didn't have as high a level, there was a coincidental chloroform spill at the same time, of near the same magnitude and I didn't believe it either until I went over the data. But, it goes to further illustrate the point that, in that instance, assuming the carbon tetrachloride was high, not only was carbon tetrachloride high, but there was also a coincidental spill.

C. SONICH: From the available monitoring data we looked at trihalomethanes and other pollutants in the drinking water, and we saw relatively low levels at the time of the spill, so I'd be interested in looking at your data.

UNKNOWN: This was found in the Huntington intake, too, so I don't know where the spill took place, but there was chloroform found in the raw water at that location—not nearly at the same concentration as the carbon tetrachloride, but then we may not have sampled at the right time.

J. F. BORZELLECA: I would like to comment on the method of administering the trihalomethanes, using gavage versus drinking water. Gavage is used for a number of reasons. One, so that we can carefully note the dose that the animal is given. You're giving him a specific amount based on body weight or surface area and it's very carefully measured and administered. Secondly, this method is used because of physical and chemical problems with material—can't get it into solution, at the concentrations required for the test. We recognize that there are differences in the kinetics of giving it all at one time versus sustained exposure when it's in drinking

water or feed. At least in our data, we don't find differences in the metabolism of the transformation. The same metabolites are formed but at somewhat different rates. Certainly, the peak blood levels are different and absorption would be faster. Most of these metabolites are absorbed quickly from the gut, so it does depend on concentration when it's administered by gavage and when it's a more sustained effect.

Some years ago, we did a study using one of the food colors, either putting it in drinking water or giving it by bolus administration. We saw effects only when administered in a bolus form and not when administered by the other two routes. There are differences using that kind of administration. The other question, also from the same individual, had to do with the effects we saw in the testes on DNA and when the material was administered intratesticularly, intraperitoneally, or by mouth—why did we not see an effect when given by mouth or why did it take so long? Fortunately for us, in both males and females, the testes or sperm is protected by a blood-testes barrier. Some materials do get through the barrier under certain conditions. The work of Dixon and Lee in North Carolina, among others demonstrates this and some of our work as well. You bypass the barrier when you administer the material intratesticularly. When it's given by mouth or intraperitoneally, you may see an effect, as we did. It took a long time, primarily because of blood flow and just inability for much of the material to get through the testes barrier. So, in fact, we are protected. When we look at the presence of materials in the serving, for example, just about anything you take by mouth would be present in the extracellular fluid late, but in very small levels. When you do distribution studies, as we did with kepone, for example, you find very little crossing the testes barrier. But if you give the materials intratesticularly, you will find a rather marked effect on sperm development and on DNA. It's then simply a matter of a protective barrier.

I'd like to note that in the Hazelton study on chloroform utilizing gavage with 250 mg/kg/day, they did not get the increase in longevity and controls we got feeding in the drinking water, so there truly are differences in the area. Concerning this business of getting chloroform in solution in the drinking water, it just takes an awful lot of mixing. It will go, but it takes a long time.

In Britain, when they used the corn oil material and tested it against an aqueous vehicle for chloroform in mice, they got substantial differences in the tumorigenicity in the kidney. So, there was a big increase when corn oil was used.

Corn oil is not without problems itself. We have found that in both the immune work that one

is familiar with and the behavioral studies, the corn oil—as pure as it appears to be—is not without effect on some of the systems.

Maltoni has done some testing with corn oil versus water with vinyl chloride. The corn oil administration produces as a great difference as when you give it by inhalation.

J. A. COTRUVO: Once we were looking into polynuclear aromatic hydrocarbon human exposure and trying to do source contribution studies. Even in the case of benzopyrene, we were finding on the order of about 50 µg/day benzopyrene in the diet and other PAHs, in addition. A substantial amount was from vegetable oil intake. I don't know how much corn oil might contribute, but that might be another confounding factor in animal studies if residual PAH levels are high in the vehicles.

UNKNOWN: What strain of mouse are you using? I think Dave showed some data that showed increased fat in the liver of the B6C3F1 mouse, and I gathered from Al's presentation that there was really nothing to be seen in your mice. I wonder if that's the strain difference.

J. F. BORZELLECA: We are using CD1—Charles River.

Two things that we've touched on—how to best spend limited resources and how to proceed that toxicologists and epidemiologists can better complement one another. I think one of the things epidemiologists have shown in the studies—and the thing we sometimes lose sight of—is the fact that only things that have shown up with any consistency have been colon and rectal cancer. I think that the question about the effect on the intestinal flora is an example of the type of experiment that could be done to take advantage of the fact that we might want to look more closely at other risk factors or what we've come to know about rectal and colon cancer, and that in using the knowledge that epidemiologists have gained in that respect in the study of those diseases, we might better allocate the funds in our research efforts.

J. M. SYMONS (U.S. EPA, Cincinnati): In spite of both your and our urging the water utilities to improve the chemical quality of their water from an overall point of view to the extent possible, most of the utilities feel that the least cost solution is the one that they will choose regardless of any peripheral effects. I wonder if you anticipate our heading towards giving any more guidance to the utilities in terms of either the control of other by-products or the use of alternate disinfectants, so that we can possibly go beyond merely the markers of THMs and on to other regulatory items that might further improve water quality?

J. A. COTRUVO: Well, I don't know. I guess what you're saying is, as a result of the conference, are

there some specific conclusions that one can draw on the various items such as chlorine dioxide or ozone and that could add to either the guidance or the regulations, and should we then busy ourselves with other things besides just disinfectants and their by-products. I find myself on the matter of chlorine dioxide in about the same situation that I was when I got here. There have been a number of studies, generally with negative results, but certainly the studies themselves haven't explored all the possibilities that have to be explored before one can make a decision that could result in very widespread exposure to the population to a new agent at high levels. I would say that the studies reported here are some of the best I have seen to date. I lean very much towards studies that examine clinical effects in humans. When they can be either shown or not shown, the level of credibility on the decision is exceptionally good, and those were kinds of studies that were done, and I think that they were very successful in the areas in which they looked.

The remaining question is, are there some other areas in which they should also look, such as with young children or other parts of the population that might be susceptible to oxidant stresses. I'm somewhat reassured from the data that were presented that there do not appear to be overt toxic effects, at least among healthy individuals that were tested with those disinfectants and their degradation products. Where that leads me is to say that we should probably continue to be cautious and still strive to minimize the unnecessary introduction of any chemical entity into drinking water, and if there is a need for that substance, such as disinfectants, take pains to eliminate as many of the side effects as possible: for example, reducing dosages as much as possible, minimizing by-products as much as possible, eliminating reactants in the water that will result in increased dosages as much as possible. It seems to me to be the common sense approach.

When one is trying to deal with a problem that has such a broad spectrum of receptors—the millions of people, and broad ranges of physical states or health—just from the mathematics of it, I think that we have to assume that there's a reasonable probability that some portion of that population is going to be somewhat more susceptible to whatever the end result would be than the average person in the population. At this point in the chlorine dioxide area, I could say we are somewhat satisfied with the data that are being presented but there are still other kinds of extensions of that work that will be done before a more definitive statement can be made.

On the rest of the situation, I don't think we're ready to make any more specific judgments beyond

what's already been made. I think that if we look back into the history of water treatment, there are some good lessons to be learned. Traditionally, simple solutions to complex problems are the best. Fortunately, there are such things in water treatment. The best example of that is chlorine, where, if one wanted to take a highly sophisticated look at waterborne disease transmission, one could have spent millions of dollars and decades of time identifying all the particular pathogens that might be in water at a particular time, devising analytical tests, and devising monitoring frequencies, or developing antidotes. The simple solution happened to be a very cheap chemical that pretty much across the board dealt with the pathogen problem. It may be that we're in the same spot when dealing with trace contaminants. I know that you as engineers, are proponents of this approach. If there's sufficient information to suggest the existence of the problem, i.e., contamination, instead of expending the resources that might be spent to further elucidate risk potential and to quantify it very precisely, it may be that the most direct approach is just to solve the problem rather than to further explore and identify it. In this case, as has been discussed, if we can't solve the problem, we can certainly minimize it by using the kinds of techniques that are available to water engineers to reduce the presence of extraneous substances in the water so that if there is a risk, that risk will have been considerably reduced. In fact, that's what we would have done ultimately anyway: had we explored the problem further and precisely quantified it, eventually then we would wind up with some engineering solution. So, one point of view is why not just apply the solution, anticipating the potential of the problem? I think that's still our philosophy—avoiding unnecessary contamination of drinking water and minimizing that contamination whenever it is feasible to do so.

But I think cheap is the operative word. If chlorine had been \$10 a pound, back in the 1900s, how they would have tackled this problem, if they wouldn't have tried a more elaborate scheme. I think the common sense approach costs money, and we're having trouble convincing the utilities to spend that money just because the likes of you and we think that it is prudent.

I thought that was one of the things that was admirable in the presentations of Zoeteman and Kool, because their approach in The Netherlands has been to look at the situation with the best handle they had and to do everything in their power to reduce whatever hazards they were able to identify. It seems as though they are able to operate there without having the absolute definition of the degree of hazard that's involved. You saw in

their presentation that they very quickly adjusted treatment conditions to lower the generation of mutagenic activity in their waters from chlorination. They changed very directly from breakpoint chlorination, but it does not quite follow that we can follow this same premise in the U.S. The country is a lot bigger. You can do a lot of things more easily in a smaller group than you can in a larger one.

Obviously, it has been shown that a wide variety of substances are introduced in drinking water as a result of disinfection processes or oxidation processes. A considerable amount of effort has concentrated on identifying those substances and has certainly shown that some of the principal ones—trihalomethanes—are ubiquitous and that there may be toxic consequences from exposure to those substances. That kind of information came primarily from animal studies at high doses. At the same time, there have been almost 30 epidemiological studies of various types that have been conducted to date with varying results, but at least some thread of relationship between variables relative to chlorination or other aspects of water quality and increased risk of cancer of certain types. A number of test systems have been devised to obtain more information on the aggregate of the chemicals in the water, given the difficulties of dealing with individual substances and the problems that one always has in identifying toxicology of individual chemicals when in fact the exposure is to a variety of chemicals simultaneously. So, the toxicology becomes especially complex.

Some very innovative work is therefore being developed in the area of concentration of water samples and test systems that can demonstrate relative effects of those concentrates as well as pure chemicals in rapid biological measurement systems. Animal surrogates for evaluating human risks have their benefits because they do allow a relatively controlled experiment. However, they have their disbenefits because they involve non-environmental dosage levels and of course necessitate the use of nonhumans as targets for the tests. So, those two heroic extrapolations have to be made whenever that kind of study is done. At the same time, there are some endpoints that can be investigated in humans, and studies have been conducted where actual human volunteers were exposed to various substances and a great number of endpoints were observed. Fortunately, at least in the studies reported, most of those appeared to be negative, at dosages similar to drinking water levels.

A great amount of technical work has been done and there is certainly the possibility of performing much more. The question we have to ask is which of that work should be done and where should the

emphasis be placed in the future to give us the best payoff in being able to arrive at decisions that have to be made on the questions of water quality and risks associated with drinking water contamination? One of the things emphasized in this conference was the matter of trying to unite some of the different disciplines in the investigations that could emphasize the benefits of each of the disciplines, such as studies of the type done at Bethesda, Ohio, even though it was a small one. It was one where the population was studied in its natural habitat by careful clinical studies and investigations; numbers were collected on those people in an attempt to try to find any endpoints that result from that exposure.

So, given the limited data—which are in one sense limited but in another sense substantial by comparison and have pretty much been developed in the last five years—we are at the point of concluding that there may be risks from exposure to substances in drinking water and that there are ways of dealing with those substances and at least attempting to reduce those risks. So, now that

we've lived through the THM era—and I think we all understand now that THM is only part of the question—we need to find out if there are ways to get through this very complex question. We must steer the research in the direction to answer specific questions. We need to decide what the question is so that we'll know it when we get to the answer. For example: which substances, which toxicological endpoints, which surrogate tests and what is their validity in representing actual human experience? So, we must ask the questions very specifically at the front end and design the research that will resolve them in the future.

I commend all of the speakers and all of the participants. I think it's been an excellent workshop and it's been a good opportunity for all of us to get together and communicate more than we ever have in the past on this subject, at least to a broader extent. All I can say is keep going; there is much to do. We need that information to make the regulatory decisions that could be very significant in terms of the health of the country.